*Journal of Helminthology, Vol.* xii, *No.* 3, *June*, 1934, *pp.* 119–122.

# On gall-formation due to the nematode *Anguillulina graminis*.

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## Introduction.

Amongst the plant-parasitic members of the genus *Anguillulina*, 12 species have been recorded from true galls and of these, one occurs on roots and the remainder on shoot structures. In four only of these species have we any detailed information on the structure of the galls formed as a result of the action of the parasite, namely in the cases of *A. tritici*, *A. agrostis*, *A. graminophila* and *A. radicicola*. In the case of the two first mentioned, galls are formed from modified flower tissues ; in the case of *A. graminophila* we have galls on leaves and in *A. radicicola* galls on roots. There are considerable gaps in our knowledge of the reaction of the host plant to the particular parasite attacking it and also concerning the mode of action of the latter in stimulating the production of galls by the host.

In view of these considerations, suitable material being at hand, an opportunity was taken of studying the structure of the galls caused by the grass infesting species, *Anguillulina graminis*, as in the writer's earlier paper on this species (1927) the structure of the galls was not investigated.

## Material.

Thanks are due to Mr. J. C. F. Fryer of the Ministry of Agriculture Plant Pathological Laboratory, Harpenden, for the few blades of grass which furnished the material for this study. They came from a tuft of grass collected on Studland Heath, Dorset and were submitted to the writer for an opinion on the species of nematode causing the galls. The latter were in a young condition and had a pale yellow colour ; later on such galls become deep reddish purple in colour. On opening a few of the galls one or two adult worms of each sex were obtained from each and these were identified as *Anguillulina graminis* (Hardy, 1850) Goodey, 1932.

The grass was readily recognisable as a species of *Festuca* but the exact species was not easy to determine since when the galls appeared in

April, there was no sign of any inflorescence on the tuft and before the time for the flowers to appear the plant had been practically destroyed by lepidopterous larvae which had been feeding on it. In these circumstances a provisional identification has been based on the fine, needle-like shape of the leaves and on the fact that a cross section of a leaf shows that the sub-epidermal ring of sclerenchyma is well developed and is practically continuous. These characters point to the grass being, in all probability, *Festuca capillata* Lam. syn. *F. tenuifolia* Sibth. (Fine-leaved Sheep's Fescue) as figured by Butcher and Strudwick (1930).

## Structure.

Fig. 1 is a drawing of a transverse section of a normal leaf cut a short distance above a gall. From this it is clear that the two sides of the upper part of the leaf are permanently folded so that a narrow groove is formed along the median line. At the entrance to this groove and lining its walls there are many spine-like hairs on the epidermis. Similar spiny hairs also occur on the outer epidermis. Immediately within the rather regular epidermis there is a strengthening layer, two or three cells deep, of small, thick walled sclerenchyma cells, which extends from just inside the mouth of the groove all the way round the outside except where it is interrupted at the several points occupied by 6 thin-walled motor cells. The substance of the leaf is composed of irregularly polygonal mesophyll cells containing chlorophyll. There is a principal vascular bundle extending along the keel of the leaf and three smaller subsidiary bundles, two on the left and one on the right. There are no air spaces amongst the mesophyll cells.

Fig. 2 is a transverse section of a gall on the same leaf as that from which the section shown in fig. 1 was taken ; it is also drawn to the same scale. It is at once clear that there is a great increase in the width of the leaf for whereas the section shown in fig. 1 is deeper than wide, the gall is wider than deep. Accompanying this there is a great increase in the amount of tissue on the upper side of the leaf on each side above the level of the vascular bundles. This has produced a bulging inwards of the tissues on either side of the groove which is in consequence flattened and laterally extended. Coupled with these gross alterations, and doubtless causally related to them, there has been considerable increase in the size of individual cells of all the tissues of the leaf. The epidermal cells, particularly in the upper region, have become much elongated and

flattened. The cells of the sclerenchyma layer, though but little enlarged on the under side of the leaf, are noticeably stretched and enlarged on

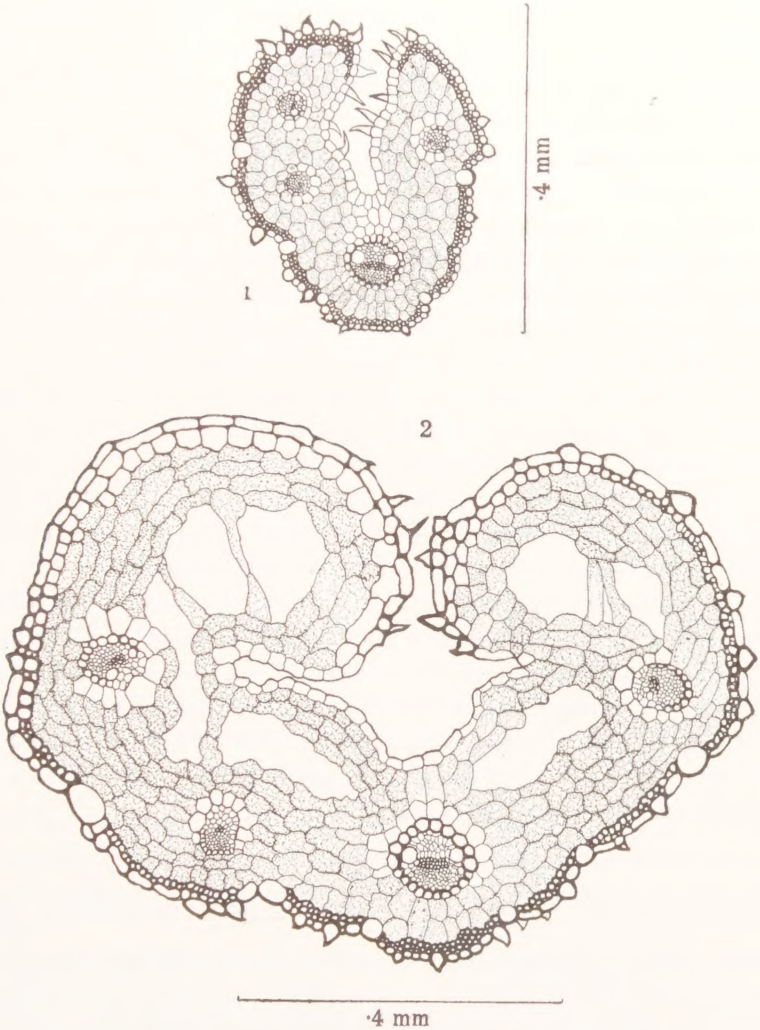


Fig. 1.—Transverse section of a normal leaf of *Festuca capillata* Lam.

Fig. 2.—Transverse section of a gall from the same leaf as fig. 1, drawn to the same scale.

the upper side, especially on the left side. The four vascular bundles are all larger than in the normal section, as are also their component elements, and finally, the mesophyll cells are all much larger than in the normal leaf. Cavities have been formed in the mesophyll a few cells of which bridge the spaces here and there. The parasites lie free in these spaces and do not enter the cells since on opening up a gall in water they can be floated out easily into the surrounding liquid and one never finds them held within cell walls.

The observations here recorded are in line with those made by the writer in two earlier papers ; one on *Anguillulina radicicola* (1932) and the other on *A. graminophila* (1933). In the case of these species also it has been shown that the presence of the parasite leads to great increase in the number and size of the cells of all the tissues involved in the gall : epidermis, cortex, endodermis and vascular elements of the root attacked by *A. radicicola* and epidermis, mesophyll and vascular bundles of the leaf attacked by *A. graminophila.* In both cases the parasites live intercellularly in cavities formed amongst the cortical or mesophyll cells and not intracellularly within the gall cells. In the present case also, as in that of the two species just mentioned, it is probable that the great increase in number and volume of the various cells composing the gall is due to the stimulus of some diffusible secretion poured out by the parasites rather than to attack on individual cells by means of the mouth stylet since tissues remote from the immediate location of the parasite become involved equally with those close to it. This view of the probable manner of causation of plant galls has already been discussed in the paper dealing with *A. radicicola* (l.c.) where it is shown to be in accord with the work of Kostoff & Kendall (1930) on root galls due to the root-knot eelworm, *Heterodera marioni.*

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# Some observations on the effect of dietary deficiency on infestation of chickens with the nematode *Heterakis gallinae*.

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There has recently been published a series of observations, based on experimental evidence, which goes to show that the course of an helmintic infestation may be significantly affected by the diet which is fed to the experimental animal. These results which have emanated mainly from Ackert and his colleagues (1929 and 1931) and from Foster & Cort (1931 and 1932), Hiraishi (1927 and 1928) and from Nagoya (1931) show that the vitamins are frequently significant factors affecting the development of Ascaridia, hookworms and Ascaris. In a later paper (1933), Ackert and others showed further that the type of amino-acids and the variety may also be factors influencing the development of *Ascaridia lineata* in chickens.

The present writer (1933) was not so successful when she used *Heterakis gallinae* in the absence of vitamin A in chickens but using the same worm in the absence, or at least the deficiency of mineral matter, she has obtained results which are strikingly successful.

## Material.

The material used corresponded closely with that used in previous experiments (1933). The chicks were sex-linked cockerels, obtained as day old birds and reared under parasite-free conditions in batteries indoors.

*Heterakis gallinae* was obtained from the caeca of chickens, kindly supplied by local poultry dealers. The eggs were cultured on the bench in the laboratory until the infective stage was reached.

## Diets.

Up to the age of 4 weeks, all the chicks were fed a good normal diet, but after this age they were put on the experimental diet. This was composed as follows :—

| | | |
|---|---|---|
| Yellow maize | ... ... ... ... ... | 50 parts |
| White maize | ... ... ... ... ... | 15 ,, |
| Tapicoa meal | ... ... ... ... ... | 35 ,, |
| 1% cod liver oil | ... | a little rubbed in daily. |

Cooked lean meat was given three times weekly.

This diet was balanced and contained abundant amounts of all the basal foodstuffs together with plenty of vitamins. It was palatable to the birds and they ate abundantly of it at first until they began to feel the effects of the deficiency. It was very low in mineral content, particularly in calcium and phosphorus. As a result of this the chicks ceased to grow though they ate well for a time. Their bones became very thin and papery and were easily cut with a knife at post-mortem. Another result of the deficiency was that the birds developed habits of cannibalism and for this reason they were penned separately.

## Experiments.

Two groups of chickens were put on the experimental diet at the age of 4 weeks. Half of these birds received an extra ration consisting of a mixture of limestone grit, oyster shell, calcium carbonate and calcium phosphate. This mixture made up the deficiency of mineral matter in the basal diet. The other chicks received nothing extra. Four weeks later, all the chicks were fed 200 infective eggs of *Heterakis gallinae* in a flour pellet. They were killed after 24 days. The caeca were examined and the numbers of worms there were removed and counted.

It was obvious without any statistical examination of the numbers, that there was a great difference between the two groups. Far more worms were present in the group fed the deficient ration than in the control group, though the size of the worms seemed not to have been affected by the deficiency. In group A, fed an adequate diet, the numbers of worms recovered from the caeca varied from 23 to 101, the mean being 50·8 worms per bird and the standard deviation 16·7. In group B, however, fed deficient minerals, the numbers varied from 47 to 119, with a mean of 81·18 worms per bird and a standard deviation of 23·8.

In a second experiment conducted in exactly the same way, a similar result was obtained. In group A the numbers varied from 6 to 93 with a mean of 39·68 and a standard deviation of 23·6 while in group B the

numbers were higher, varying from 45 to 127. The mean here was 80·95 with a standard deviation of 22·6.

| | Numbers of *H. gallinae* in caeca varied from | Mean | Standard Deviation | |
|---|---|---|---|---|
| Chicks fed adequate minerals | 23 to 101 | 50·80 | 16·7 | Expt. 1 |
| ,, deficient ,, | 43 to 119 | 81·18 | 23·8 | |
| Chicks fed adequate minerals | 6 to 93 | 39·68 | 23·6 | Expt. 2 |
| ,, deficient ,, | 45 to 127 | 80·95 | 22·6 | |

These figures were closely examined by statistical methods and the differences were found to be highly significant. As all the factors, other than the mineral content of the diet, were controlled, one is able to say that the deficiency of the mineral matter has significantly affected the fate of the *Heterakis gallinae*.

One fact, in connection with the constitution of the diets should not be overlooked. There was a deficiency of both calcium and phosphorus, though there was a complete absence of neither. It is not easy to obtain a diet suitable for chickens in which one particular mineral constituent is deficient without making it very unpalatable and unsuitable. The writer, therefore, felt that it was better to have the diet as constituted than to have it unpalatable. Had it been so, another factor would have crept in which would have been difficult to control for the birds would have refused the food and would probably have died of starvation.

## Conclusion.

The mineral matter of the diet significantly affects the fate of *Heterakis gallinae* infestation in chickens.

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*Journal of Helminthology, Vol.* XII, *No.* 3, *June,* 1934, *pp.* 127–136.

# The morphology and the systematic position of a new Trematode from the intestine of the golden orfe, *Leuciscus idus*, with a note on the classification of the family Allocreadiidae

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A considerable amount of attention has been paid to the parasites of fresh water fishes in the West on account of the great economic importance of fishes, particularly their value in food. Their importance further increases, when we find that they also serve, in several cases, as carriers of the larval stages of the helminth parasites of man and domesticated animals. The work prior to 1924 has been summarised by Nicoll (1924), where he has given a complete bibliography on the subject, and has, besides, indicated the important and characteristic Trematodes of fresh water fishes. In this list he enumerates the forms like *Sphaerostoma bramae* (= *Dist. globiporum*) ; *Allocreadium isoporum* ; *Catoptroides macrocotyle* ; *Azygia lucii* ; *Bucephalus polymorphus* ; *Diplostomum volvens* ; *Diplozoon paradoxum* ; and *Gyrodactylus elegans*. This list has been considerably enlarged by subsequent workers by the addition of several genera and species from the fresh water hosts from different parts of the world, the principal workers being Nicoll, Odhner, Poche and Travassos. In India, the additions to our knowledge of the Trematode parasites of fishes has been made by Southwell (1913), Southwell and Prashad (1919), Verma (1927) and Thapar (1930). The present paper adds yet another genus to the already extensive list of Trematode parasites of fishes.

The form described in the present communication, while agreeing with the genus *Sphaerostoma* in general characters, differs from it in certain important features that necessitate the creation of a new genus, *Cotylogonoporum*, for its reception. This new genus, as will be seen, is interesting in so far as it illustrates certain important features of systematic nature that offer grounds for the revision of the family Allocreadiidae.

COTYLOGONOPORUM ORFEUM, N.G., N.SP.

*Cotylogonoporum orfeum* is a small trematode found in the intestine of the golden orfe, *Leuciscus idus*. It is 2·1 mm. long, with a maximum diameter of 0·65 mm. in the region of the acetabulum, and is rounded at either end. The surface of the body is smooth, being devoid of spines.

The oral sucker is circular and subterminal and is 0·2 mm. in diameter. The acetabulum is larger than the oral sucker, being twice the size of the latter and is 0·4 mm. in diameter. It is situated at a distance of 0·65 mm. from the anterior end.

The genital pore lies on the ventral surface between the oral and the ventral sucker, slightly to the right of the median line, just anterior to the point of bifurcation of the oesophagus into the intestine, 0·3 mm. in front of the acetabulum. It is surrounded by a strong circular genital sucker, 0·13 mm. in diameter. This is a very characteristic feature of the present genus and, in this respect, appears to connect it with the members of the family Heterophyidae with a genital sucker.

The excretory pore is terminal, situated at the posterior end of the body. It leads internally into an elongated bilobed excretory bladder, 0·2 mm. long. Anteriorly, it leads into two lateral longitudinal ducts, one on either side.

The alimentary canal begins at the anterior end at the mouth, which is a transverse slit in the centre of the oral sucker. It leads into a pear-shaped muscular pharynx, 0·1 mm. long by 0·12 mm. broad. Then comes a wide oesophagus 0·21 mm. long. The latter bifurcates posteriorly into two simple intestinal crura that run back to the posterior end of the body.

The male reproductive organs consist of two testes and their ducts. The two testes are situated behind the ventral sucker and are widely separated from each other by ovary and other female genital organs. Each testis is a trilobed structure, with rounded outer and posterior margins. The anterior testis is situated just behind the ventral sucker, slightly to its right and is 0·3 mm. long by 0·15 mm. broad. The posterior testis is larger than the anterior and lies in the last quarter of the body. It is 0·35 mm. long and 0·25 mm. broad.

The cirrus sac is a large cylindrical sac, 0·3 mm. long. It extends from the anterior end of the ventral sucker forwards where it is slightly

curved to the right. It contains within it (1) the vesicula seminalis; (2) pars prostatica; and (3) a muscular cirrus.

The vesicula seminalis is a transversely elongated broad sac, lying at the posterior end of the cirrus sac, and filling up the curved portion of the sac. It is 0·1 mm. long and 0·07 mm. broad. Anteriorly, it is constricted and opens into a sac-like pars prostatica, about 0·08 mm. long. This leads by a short narrow duct into an elongated muscular cirrus, about 0·14 mm. long. This latter organ opens at the genital pore in the centre of the genital sucker, already mentioned. A large number of unicellular glands open into the pars prostatica and fill up the entire space in the cirrus sac round the pars prostatica.

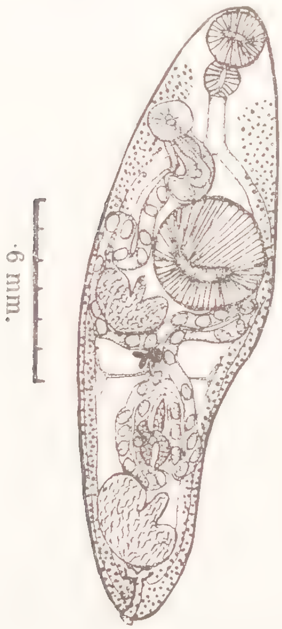



Fig. 1.—*Cotylogonoporum orfeum*, n.g., n.sp., entire worm, ventral view.
Fig. 2.—Cirrus sac, showing the contained structures, greatly enlarged.

The female organs consist of an ovary and its duct, together with a number of accessory organs associated with it. The ovary is a single, deeply lobed organ, lying between the two testes, slightly to the left of the median line. It consists of four lobes, arranged crosswise, each lobe being very much elongated. It is 0·3 mm. behind the ventral

sucker and about 0·09 mm. in front of the posterior testis. From the point of division of the lobes arises anteriorly a forwardly running oviduct, which, at about 0·15 mm. in front of the anterior lobe of the ovary receives a duct from the receptaculum seminis.

The receptaculum seminis is a pear-shaped organ, 0·05 mm. long, situated between the ovary and the anterior testis. The main duct then runs forward to the öotype, which is marked by the aggregations of shell glands. It also receives the duct from the cylindrical yolk sac, 0·06 mm. long. Laurer's canal has not been observed in any of the specimens, but it may possibly be present in the same position as in the genus *Sphaerostomum*.

The vitelline glands are very extensive, consisting of numerous small follicles, situated laterally on either side of the body mainly extra-caecal, but partially overlapping the caeca both dorsally and ventrally. They extend from the pharynx in front to the posterior end of the body, behind the posterior testis, where they also occupy the central portion. A vitelline duct on either side of the body opens into a transverse vitelline duct in front of the ovary. The two transverse ducts unite to form a common duct that leads to the öotype. Just before entering the öotype, the common vitelline duct is joined by an enlarged yolk sac reservoir.

The uterus arises from the left of the öotype. It follows a very characteristic course within the body. It runs backwards from the point of its origin to the right side of the ovary as far back as the posterior testis. It then curves forwards, thus doubling on itself on the same side, to the front end of the ovary. Here it curves back to form a similar loop on left of the ovary and then runs forward to the ventral sucker. Here it takes a sinuous course transversely across the body to the posterior end of the anterior testis and ventral to the intestinal caecum of the right side and finally runs forward along the right side of the cirrus to open at the genital sucker, in front of the male genital pore.

The eggs are operculated, yellowish-brown in colour and provided with thick shells. They are oval and measure 75–80 $\mu$ long by 45–50 $\mu$ broad.

The distinguishing characters of the genus *Cotylogonoporum* may be summarised thus :—

1. Small size of the body, with smooth skin devoid of spines.
2. Presence of the genital sucker in front of the acetabulum.

3. Inter-testicular position of the lobed ovary.
4. Peculiar position of the lobed testes.
5. Peculiar disposition of the uterine coils between the acetabulum and the posterior testis.
6. Peculiarities of the öotype complex ; and
7. Operculated eggs.

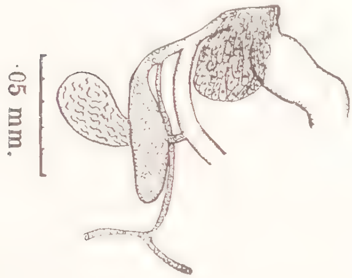



Fig. 3.—Öotype and associated structures in *Cotylogonoporum orfeum*, semi-diagrammatic.

Fig. 4.—Eggs of *Cotylogonoporum orfeum*.

The genus *Cotylogonoporum* resembles the genus *Sphaerostomum* in the general shape of the body ; the absence of the body spines ; the relative sizes of the two suckers ; and the inter-testicular position of the ovary. It differs, however, from *Sphaerostomum* in the following characters :—

1. The presence of the genital sucker ;
2. Lobed ovary and testes ;
3. The position of the genital pore ;
4. The shape of the cirrus sac and the seminal vesicle ;
5. The presence of a distinct yolk sac ; and
6. The peculiar disposition of the uterine coils between the acetabulum and the posterior testis.

These characters appear to us to be sufficient to justify the creation of a new genus, *Cotylogonoporum orfeum*, for these trematodes.

## Discussion on the family Allocreadiidae.

In the foregoing description, it has been shown that the genus *Cotylogonoporum* resembles in its important features the genus *Sphaerostomum* and should, therefore, be classed along with the latter under Sphaerostomatinae Poche, 1925. We shall consider the status of this subfamily later on.

Nicoll (1909) described several trematodes of the family Allocreadiidae from fishes and discussed their systematic position, with the result that he created a new subfamily Stephanophialinae, on the characters of possessing a circumoral collar with tentacular papillae and the possession of a short prepharynx.

The genus *Sphaerostomum* was kept unclassified. Neither Lühe (1911) nor Nicoll (1915) included it in their systems of classification. Poche (1925) was the first to recognise it in the system and created a new subfamily Sphaerostomatinae for its reception. This subfamily was placed by Poche under the family Allocreadiidae which he divided into five subfamilies thus :—

(*a*) Allocreadiinae Looss, 1899.
(*b*) Lepocreadiinae Odhner, 1905.
(*c*) Stephanophialinae Nicoll, 1909.
(*d*) Sphaerostomatinae Poche, 1925 ; and
(*e*) Coitocaecinae Poche, 1925.

Poche has given an elaborate description of the subfamilies and has further given his arguments for the inclusion of all under the family Allocreadiidae. Fuhrmann (1928) has subsequently adopted this classification for the group, but has rightly excluded Coitocaecinae from it placing the genus *Coitocaecum* under the family Opecoelidae. To this view we fully subscribe. The subfamilies Lepocreadiinae and Stephanophialinae resemble the subfamily Allocreadiinae in all essential features, i.e., the position of the genital pore, the structure and composition of the cirrus pouch ; the position of the testes ; the extent and situation of the uterus ; and the condition, size and number of ova. In all these, the uterus never extends beyond the anterior border of the first (anterior) testis and the excretory bladder is a simple straight tube. These characters fully justify their inclusion under the family Allocreadiidae. But the case is different with the subfamily Sphaerostomatinae, and the writers consider that the inclusion of this subfamily under the family Allocreadiidae is not justified. This is further strengthened, when we consider the general topography of organs in *Sphaerostomum* and *Cotylogonoporum* on the one hand and of the members of the other subfamilies of Allocreadiidae on the other. We have shown above that the members of the other subfamilies of Allocreadiidae resemble each other in all essential characters, but the case is different with the subfamily Sphaerostomatinae.

This latter differs remarkably from the other subfamilies in the following features :—

" The excretory bladder here is bilobed in front, the testes are separated from each other by a considerable space, the ovary is situated in between the two testes, and the uterus reaches as far back as the posterior testis." With regard to this Poche (1925, page 161) states as follows :—

" Die Gattung *Sphaerostoma* hatte bisher überhaupt noch keinen Platz im modernen System der *Digenea* angewiesen erhalten. Sie fällt mit fast allen Charakteren durchaus in den Rahmen der Familie Allocreadiidae. Als Abweichungen sind nur zu erwähnen : dass die Exkretionsblase an ihrem Vorderende zwei allerdings sehr kurze Zipfel ausweist, was sonst bei dieser Familie nicht der Fall ist ; dass die Hoden nicht wie sonst bei dieser (median oder schräg) dicht oder höchstens (*Podocotyle atomon* (Rud.) [s. Odhner, 1905, Tab. II, Fig. 9] in geringer Entfernung hintereinander liegen, sondern durch einen beträchtlichen Zwischenraum voneinander getrennt sind und der vordere Hoden vor dem Keimstock liegt statt hinter ihm wie bei den meisten Allocreadiidae oder wenigstens neben (" und mehr oder weniger " hinter) . . . dass der Keimstock weiter hinten liegt als sonst bei den Allocreadiidae, wobei jedoch der Unterschied gegenüber dem bezüglichen Verhältnis bei *Allocreadium labri* (cf Odhner, Fig. c.) nicht gross ist ; und dass endlich im Zusammenhange mit der anderen Lage des vorderen Hodens der Uterus nicht wie sonst bei unserer Familie höchstens bis zum vorderen, sondern bis zum hinteren Hoden nach hinten reicht. Alle diese Unterschiede sind also keineswegs derart, dass sie uns berechtigen würden, *Sphaerostoma* von den Allocreadiidae auszuschliessen. Der wesentlichste von ihnen ist die abweichende Lage des vorderen Hodens im Verhältnis zum Keimstock. Aber auch darauf könnte eine solche Ausschliessung selbst dann nicht gegründet werden, wenn *Allocreadium labri* nicht, wie wir eben gesehen haben, in dieser Hinsicht einen Übergang zwischen den anderen Allocreadiidae und *Sphaerostoma* bilden würde. . . . Immerhin sind aber die Differenzen zwischen *Sphaerostoma* und den anderen Allocreadiidae derart, dass es erforderlich ist, für jenes eine eigene Unterfamilie Sphaerostomatinae, sf. nov., aufzustellen."

It would thus appear that Poche (1925) entirely disregards the diagnostic value of the principal features, ordinarily taken into account in our system of classification of the Digenetic Trematodes. A point on

which Poche lays great stress is the existence of a form, *Allocreadium labri*, which, according to him, is an intermediate form between Allocreadiidae and Sphaerostomatinae, and therefore, he advocates the fusion of the two under a common family. But it cannot be supported by facts. If we examine carefully the structure of the forms from the two groups under consideration, we would realise that it requires a great deal of imagination to connect them through *Allocreadium labri*. The position and shape of the excretory bladder is different in the two cases. It is simple and straight in all the members of the family Allocreadiidae, but it is bilobed in the two genera—*Sphaerostomum* and *Cotylogonoporum*—of the subfamily Sphaerostomatinae. This character alone appears to us to be sufficient to isolate Sphaerostomatinae from the family Allocreadiidae. Further, the separation of the two testes from each other and the intervention of the ovary in between them in *Sphaerostomum* and its ally is not easy to derive from a condition where ovary is in front of both the testes, as is the case in the members of the family Allocreadiidae. The inter-testicular position of the ovary in Sphaerostomatinae should bring it nearer to Clinostomidae than to Allocreadiidae. Again, the disposition of the uterus is also different. In Allocreadiidae the uterine coils extend up to the anterior testis only, but here in Sphaerostomatinae they extend further back to the posterior testis. This would mean pulling the uterus back from its normal condition in the family Allocreadiidae. The position of the genital pore is also different in the two cases under review. All this shows that there are considerable differences between them and that the general topography of organs in Sphaerostomatinae is quite different from that of the members of the family Allocreadiidae. This leads us to the conclusion that there appears to be no justification for fusing them together under a common family Allocreadiidae.

The only similarities that exist between Allocreadiidae and Sphaerostomatinae are in the general shape of the body, the smoothness of the skin and the simplicity of the intestinal caeca. These characters are present in widely different families of the Digenetic Trematodes and hence no reliance can be placed on them in our system of classification. Such a course of action would lead to a considerable confusion.

The differences outlined in the foregoing paragraphs appear to us of far greater importance than the resemblances, and are based on the

characters that are generally recognised for the purposes of family diagnosis, particularly the character of the excretory bladder. Hence we venture to suggest that the subfamily Sphaerostomatinae be removed from the family Allocreadiidae and that it be given the status of a distinct and independent family Sphaerostomatidae nom. nov. to include, for the present, only two genera *Sphaerostomum* and *Cotylogonoporum* gen. nov. The characters of this new family may be summarised thus :—

" Small or medium-sized distomes, with smooth body, without spines or scales ; both oral and ventral suckers well-developed, the ventral sucker larger than the oral, nearly twice the size of the latter ; the oral sucker sub-terminal ; pharynx and oesophagus both present ; intestinal caeca simple, extending to the posterior end of the body. Testes simple or lobed, separated by the ovary, which may also be simple or lobed. Cirrus sac well developed, extending to the ventral sucker ; Seminal vesicle, pars prostatica and cirrus present ; genital opening anterior to the ventral sucker, median or lateral, with or without genital sucker. Uterus between the posterior testis and the genital opening forming a few ascending and descending coils on either side of the ovary Receptaculum seminis in front of the ovary ; Laurer's canal present ; yolk glands diffused extending from the pharynx to the posterior end of the body. Eggs large operculated or non-operculated, yellowish brown in colour. Excretory bladder bilobed. Parasites of the digestive tract of fishes."

The two genera of the family Sphaerostomatidae are distinguished thus :—

1. Genital sucker absent, testes and ovary simple, round.
*Sphaerostomum.*
2. Genital sucker present, testes and ovary lobed.
*Cotylogonoporum.*

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*Journal of Helminthology, Vol.* XII, *No.* 3, *June,* 1934, *pp.* 137–142.

# A minimal computation of the amount of blood removed daily by *Haemonchus contortus*.

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IT has been generally held that the severe anaemia and other pathogenic effects of infestation by hookworm in man and dogs, and by *Haemonchus contortus* in sheep, are insufficiently explained by the amount of blood removed by the parasites. The action of haemotoxins has therefore been postulated.

Haemolysins have been extracted from hookworms by many people, but it is still an open question whether they are injected into the hosts. The difficulty of accepting haemolysis as a cause of the anaemia is, as de Langen (1922) has pointed out, that there is no haemoglobin nor excess of bilirubin in the plasma in ankylostomiasis. Extracts of *H. contortus* were found by Brumpt and Joyeux [quoted by Brumpt (1927)], and by Schwartz (1921) to possess feeble haemolytic powers, but Fourie (1931), who has made an extensive study of the haematology and pathology of haemonchosis in sheep in S. Africa, concludes that neither haemolysis nor disturbance of the blood-forming tissues is the cause of the anaemia, and that the complete blood picture can be produced by periodic large withdrawals of blood.

The discovery by Loeb and Smith (1904), afterwards confirmed by Flu (1922) and Chandler (1929) that an anti-coagulant can be extracted from the anterior ends of hookworms is, if applicable to *H. contortus*, significant, for Hall (1920) observed that these worms move about in the abomasum, making a number of punctures which continue to leak blood. In this case, the amount of blood ingested by the worms may be a small proportion of the total loss.

The total amount of blood withdrawn daily by the worms can only be guessed, but a *minimal* estimate of the blood consumed daily by a female *Haemonchus* can be arrived at from the number of eggs laid and the amount of phosphorus they contain, if we assume that the only source of phosphorus is the blood of the host. The total phosphorus

in sheep's blood is round about 20 mg. per 100 c.cm., so for each milligram of phosphorus in the eggs laid, 5 c.cm. has to be consumed by the females even if they transferred the whole of the phosphorus ingested to their eggs. Such efficiency is improbable. Moreover, this minimal estimate leaves out of account the blood removed by the male worms.

*S* 0. Total number of adult ♀ *H. contortus* on autopsy—293.

| Date. | Total eggs in 24 hours | Eggs per adult female per day | Average daily egg production |
|---|---|---|---|
| 1.11.30 | 943,000 | 3,220 | 5,280 |
| 7.11.30 | 2,279,000 | 7,770 | |
| 12.11.30 | 1,590,000 | 5,420 | |
| 14.11.30 | 1,400,000 | 4,770 | |

*S* 46. Total number of adult ♀ *H. contortus* on autopsy—280.

| Date. | Total eggs in 24 hours | Eggs per adult female per day | Average daily egg production |
|---|---|---|---|
| 5.11.30 | 1,743,000 | 6,193 | 5,656 |
| 12.11.30 | 1,200,000 | 4,280 | |
| 14.11.30 | 1,120,000 | 4,000 | |
| 19.11.30 | 2,295,000 | 8,010 | |
| 21.11.30 | 1,627,000 | 5,800 | |

*S* 110. On autopsy 24/5/32 was found to harbour 54 ♀ *H. contortus*, 8 ♀ *Oe. columbianum* and 50 ♀ & ♂ *Ostertagia spp*. Culture of faeces from this sheep gave 96% *H. contortus* larvae, as would be expected from the much higher egg production of the species.

| Date | Total eggs passed by *H. contortus*—96% of total | Eggs per adult female per day | Average daily egg production |
|---|---|---|---|
| 15.5.32 | 526,300 | 9,700 | 10,500 |
| 16.5.32 | 481,650 | 8,900 | |
| 18.5.32 | 698,750 | 12,900 | |

*S* 178. Pure infection with *H. contortus*. Number of adult females on autopsy—1,427.

| Date | Total eggs passed per day | Eggs per adult female per day | Average daily egg production |
|---|---|---|---|
| 21.4.32 | 8,760,000 | 6,000 | 5,950 |
| 22.4.32 | 7,257,000 | 5,100 | |
| 23.4.32 | 7,200,000 | 5,000 | |
| 26.4.32 | 10,826,000 | 7,700 | |

## The Daily Egg Production by *Haemonchus contortus*.

By the method of Stoll (1929) the number of nematode eggs per gramme of faeces can be determined with not more than 10% of error. From this and the total weight of faeces passed, the number of eggs laid per day may be calculated. The egg production per female worm is computed after autopsy by dividing the estimated total number of eggs passed in 24 hours by the number of female worms present. In cases where the host is infested by one or more species, an approximation to egg production per female worm of each species may be made by counting the eggs of each species present, where such a specific determination is possible, and dividing the corresponding proportion of the total eggs passed in 24 hours by the number of female worms of either species. Where specific determination of eggs is not reliable, the proportion of larvae of the several species found in faecal cultures may be utilised instead.

The egg production of *Haemonchus contortus* varies considerably from day to day. Veglia (1928) gives a daily graph of egg production over one year which illustrates this variability.

The number of eggs laid daily by the different nematodes of ruminants varies greatly, *H. contortus* having a high, and *Trichostrongylus* spp. and *Ostertagia* spp. a very low egg-producing capacity. Below are given some figures for daily egg production over brief periods for *H. contortus* in experimentally infested lambs during the period of high egg-production occurring from the sixth to twelfth weeks after infestation.

It was not unusual for counts of over 5,000 eggs per day per female worm to be made. In the case of S 0, S 46 and S 110 there were relatively few worms present, which might be considered to influence the egg production if, as has been found by Sarles (1929) for *Ancylostomum caninum*, egg production varies inversely as the number of worms present. In the case of S 178, however, which proved to have a fatal infestation, over 1,400 female worms were present, or a total including males of 2,840 worms. It is seen, therefore, that in cases of fatal infestation egg-production may reach an average of more than 5,000 per day.

## Collection and Enumeration of the Eggs.

In order to obtain a sufficient number of eggs for phosphorus determination, for which at least 250,000 eggs were desirable, experimental lambs were infested heavily with pure cultures of *Haemonchus contortus*

larvae. In certain of the cases other helminths were present in addition to *H. contortus*, but always in less than 1% of the total eggs passed, as determined by culture and examination of larvae.

From 20 to 50 grammes or even more of faeces from such lambs were collected and triturated thoroughly in a mortar. Some 20 grammes at a time were then taken, and thoroughly broken down with 500 c.cm. of Sheather's sugar solution (sugar 50 gm. water 50 gm.), and then centrifuged for three minutes at 1,500 revolutions per minute. Following centrifugation in such cases large numbers of eggs floating on the surface of the solution in the centrifuge tube would be visible macroscopically as a white scum.

A few of the superficial centimetres of the solution were pipetted off, diluted with a considerable volume of water, centrifuged and washed repeatedly to free them as far as possible from water-soluble contaminants and floating débris. In this manner a considerable volume of eggs could be collected nearly free from contamination. After the final concentration, a measured volume of water was added to the eggs, and after thorough mixing at least three samples of known volume were drawn off with a graduated pipette, and the eggs in each sample counted separately, using a binocular microscope with low power objective. Usually little difficulty was experienced in obtaining counts which showed less than 10% divergence from the mean. Where the divergence was greater than this, a fresh series was counted.

The approximate total number of eggs collected was determined by multiplying the estimated mean number of eggs per unit volume measured, by the number of such volumes in the total suspension.

## Determination of Phosphorus in the Eggs.

The eggs were allowed to settle and the sediment washed into a silica basin, and evaporated to dryness after the addition of a few drops of 10% $Mg(NO_3)_2$. They were then heated to dull redness in an electric muffle. The ash was dissolved in dilute $H_2SO_4$ and boiled to convert pyro-phosphate to phosphate, and the phosphorus determined colorimetrically by the method of Fiske and Subbarow (1925).

The estimation of phosphorus in the samples was as follows :—

| Sample No. | Estimated number of eggs | m.g. P | m.g. P per $10^6$ Eggs |
|---|---|---|---|
| 1 | $3 \cdot 4 \times 10^5$ | 0·280 | 0·82 |
| 2 | $2 \cdot 75 \times 10^5$ | 0·218 | 0·79 |
| 3 | $5 \cdot 6 \times 10^5$ | 0·256 | 0·46 |
| 4 | $10 \cdot 4 \times 10^5$ | 0·458 | 0·45 |
| 5 | $6 \cdot 7 \times 10^5$ | 0·296 | 0·44 |
| 6 | $3 \cdot 34 \times 10^5$ | 0·840 | 0·66 |
| 7 | $4 \cdot 48 \times 10^5$ | | |
| 8 | $4 \cdot 81 \times 10^5$ | | |
| Total all samples | $4 \cdot 15 \times 10^6$ | 2·35 | 0·57 |

In the case of Samples 1 and 2, the eggs were not so well freed from vegetable débris, and this may account, in part, for the higher estimates.

## Estimate of Minimal Blood Intake Required for Egg Production of *Haemonchus contortus*.

The average phosphorus content of *H. contortus* eggs found is 0·57 mg. per $10^6$ eggs The daily egg production per female during the periods of high egg-production may average 5,000 eggs per day At such a rate of egg-production, 1,000 females would produce $5 \times 10^6$ eggs per day, of which the phosphorus content would be approximately 2·85 mg. Assuming that the phosphorus content of the egg is derived wholly from the blood of the sheep, and taking the total phosphorus content of this as 20 mg. per 100 c.cm., the worms would have to ingest at least 14·5 c.cm. of blood per day for their egg production, assuming 100% efficiency in utilisation of the phosphorus in the blood. Basing our estimate on this intake alone, we can form some idea of the severity of the blood loss occasioned by heavy infestations with *H. contortus*. It is not rare to find natural infestations of aged sheep in which 4,000 worms are present, of which roughly 50% are females. The blood required to furnish sufficient phosphorus for the egg production of these 2,000 females would be a minimum of 29 c.cm. per day. The males, too, suck blood, though probably less than the females, but we have no means of arriving at their requirements.

The above estimate of a possible loss of 30 c.cm. blood daily is only a minimal one. It assumes that all the phosphorus ingested goes into the eggs. It disregards the amount removed by the males. It does not allow for leakage from punctures nor for the parasites sucking blood in a spirit of wantonness. It is probable, however, that, like *Ancylostoma*

*caninum*, which Wells (1931) has shown actually to pass through its alimentary canal 0·8 c.cm. of blood in 24 hours, *Haemonchus* ingests far more blood than is needed for its maintenance and egg-production.

We suggest that this minimal estimate may safely be doubled and that the effects of infestation by *H. contortus* may be explained by the continual loss of blood, without the necessity for assuming any additional action of toxic secretions or products of the parasite's metabolism. How much blood a sheep can lose daily without exceeding its capacity to replenish it we do not know. It will depend on how the animal is fed. This subject is being investigated.

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*Journal of Helminthology, Vol.* XII, *No.* 3, *June,* 1934, *pp.* 143–164.

# Field experiments on the Immunity of Lambs to Parasitic Gastritis caused by a mixed infection of Trichostrongylid Nematodes.

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The following series of observations were made to investigate the nature of the resistance which lambs approaching maturity are known to develop towards parasitic gastritis caused by a mixed infection of trichostrongylid worms.

In the course of a preliminary experiment carried out in 1931 an observation was made which gave clear indication of the power of the immunity developed by lambs to parasitic gastritis. Two lambs were kept on a small plot of pasture to increase the ostertagia infection of the ground in connection with experiments on the longevity of the larvae of those worms ; and after the lambs had been there for some two months they were joined by two others, in order to increase the infestation of the ground still further. Both pairs of lambs had been reared in the buildings, free from trichostrongylid worms, and all were of about the same age. At the time of placing the second pair on the experimental plot the first pair were in rather poor condition, but they appeared to be withstanding the severe reinfestation to which they were exposed : although the second pair were in good condition at this time they soon began to show symptoms of parasitic gastritis and died within less than five weeks of their first exposure to the infection. The first pair, however, remained alive throughout the remainder of the summer, grazing on the same plot of ground ; thus showing that they had acquired a marked resistance to the parasites, or to the effects of their presence.

Field experiments of this kind have certain disadvantages in that some of the conditions are not controlled, but they hold a very important place in the investigation of the helmintic diseases of grazing animals as they provide for all the various factors concerned in the natural development of the disease (some of which may be eliminated in the scientific or laboratory test), and observations which are made under these conditions are more likely to be directly applicable to natural outbreaks of disease as they occur on farms than those made under artificial conditions.

A field experiment was therefore planned, to find out whether the chance observations to which reference has just been made might be confirmed, and to give more detailed information on the nature of the immunity.

## Plan of the Experiment.

*The Ground.*

The experimental pens were constructed on infected permanent pasture which had been grazed for some years by horses, cattle and sheep. For the successful demonstration of the development of immunity it was considered essential that parasitic gastritis should appear in some of the lambs, and crowded grazing conditions were therefore arranged. For this purpose the ground was divided into three plots, each one-third of an acre in area. Ewes were turned into the plots to keep the grass short before the experiment began ; and all the experimental lambs were confined to each plot in turn, until the grass had become very short, with the idea of forcing them to graze as near to the ground as possible, this being an important factor in the production of parasitic gastritis. Only a small ration of concentrated food was allowed to them (once daily as much as they would clear up before leaving the trough) so that they were forced to spend a large proportion of their time in grazing.

*The Lambs.*

The experimental lambs were born in the farm buildings from ewes which had been washed before entering the pens ; these had previously been washed and scrubbed and were subsequently thoroughly cleaned twice weekly. By these precautions the lambs were kept free from all but strongyloides infection.

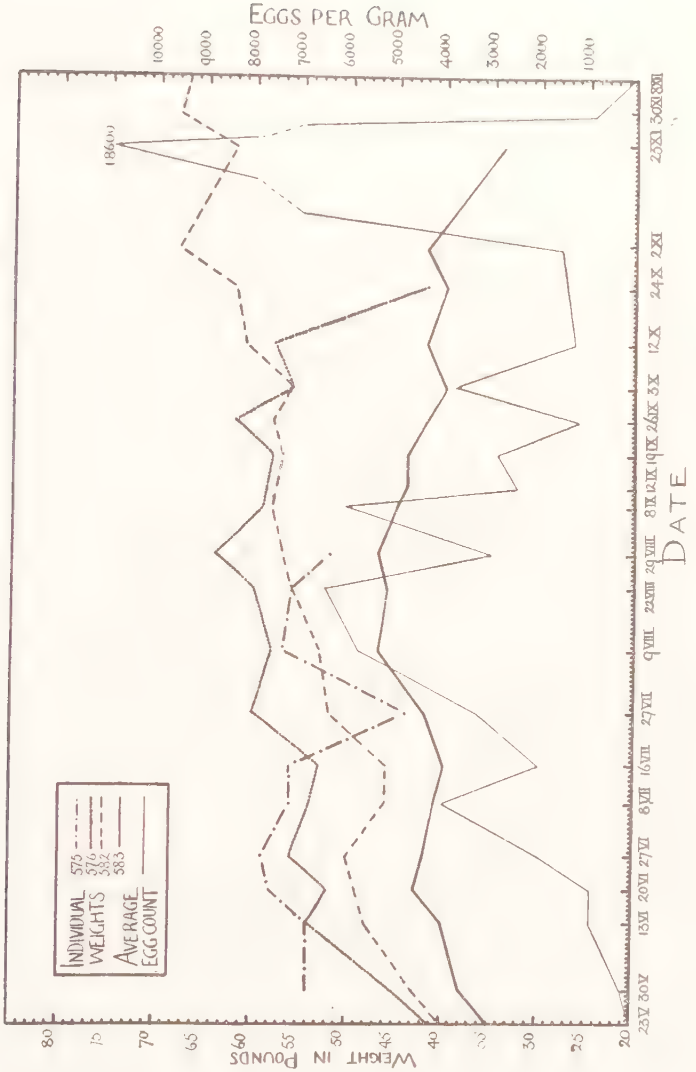
These clean lambs were divided into four groups of four lambs each, the groups being placed consecutively on the experimental plots at various times throughout the summer. There was no separation on the plots, however, all the lambs being kept together in one group in order that they might be subjected to the same conditions of infestation throughout the whole experiment.

*Method of following the Course of the Disease, and the Progress of Infestation.*

The course of the disease was followed by weekly weighings of the lambs, and the progress of infection by weekly egg counts which were made on a composite sample of faeces from each group. This was carried out by making a thorough mixture of equal amounts of faeces from each member of the group and estimating the number of eggs per gram, by a standard dilution technique. The various survival periods of the lambs also gave an indication of the relative severity of the disease in each group, Post-mortem examinations were carried out after the lambs had succumbed to the disease, or at the end of the experiment, and an estimate made of the numbers of the various species of tichostrongyles in the fourth stomach and small intestine. For this purpose the worms were freed from as much of the ingesta as possible by washing and sedimenting, and then placed in clean water in a large bowl and the volume brought up to 4,000 c.c. While the whole of this was rapidly stirred (to keep all the worms in as even a suspension as possible), 40 c.c. was withdrawn by means of a large pipette, having a funnel shaped extremity and fitted with a rubber bulb. All of the worms in the 40 c.c. were then counted ; the larger ones being picked out one at a time under the naked eye, and the smaller ones under the low power dissecting microscope.

The estimation of the numbers of worms belonging to the various species was carried out in three stages ; the desirability of going into this detail not having been at first appreciated, and necessitated working over the material three times. A count was first made with the object of estimating the total number of trichostrongylids, and for this purpose numerous slide and coverslip preparations were made of the worms from each lamb, care being taken by frequent mixing to have as representative a sample of the whole as possible. These slides were then worked over in the usual systematic manner until 50 worms had been examined, and note made as

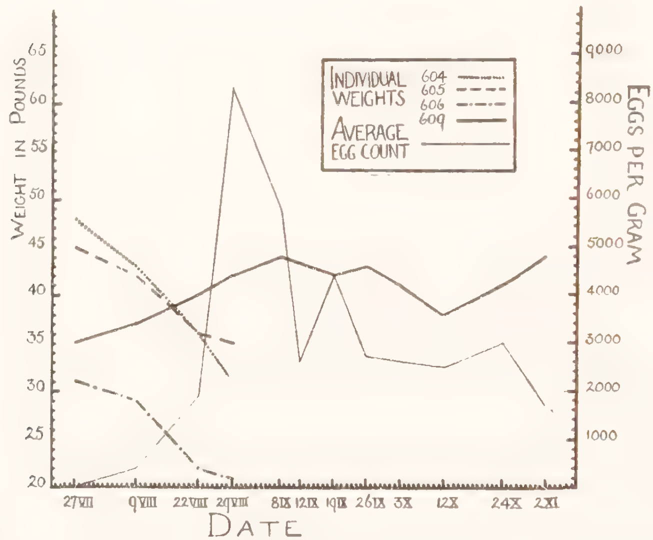

*Graph* 1.—Giving particulars of the average numbers of eggs per gram of faeces in group 1, and the weights of the individual lambs in the group.




*Graph* 2.—Giving particulars of the average number of eggs per gram of faeces in group 2, and the weights of the individual lambs in the group.

to whether they were trichostrongylids or strongyloides ; the total worm count subsequently being divided according to the proportion so obtained.

While working over these slides it was noticed that there were some marked differences in the kind of infestation present in the various lambs, and a second count was made in the same way, 50 more worms being examined from each lamb and the total number divided proportionately.

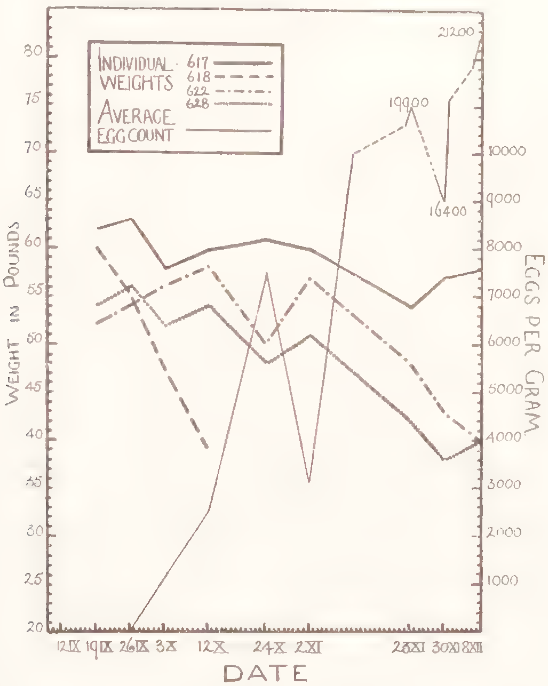



*Graph* 3.—Giving particulars of the average number of eggs per gram of faeces in group 3, and the weights of the individual lambs in the group.

Unfortunately some of the worms were destroyed after this differential count had been made and the third count, concerning the numbers of larval worms is not complete for all the groups ; this was obtained in the same way by finding the proportion of larvae present in the first 50 worms from a fair sample of the whole.

While the counts obtained in this way cannot be regarded as accurate, the resulting figures may be taken as a sufficiently reliable indication of the nature of the in'estation in the lambs, for the purpose of comparing the various groups.

The details of the observations are recorded in the four graphs and two tables which follow. The graphs are set out in a uniform way so that the dates correspond along the bottom line and make comparison more easy. Each graph shows the rise and fall in the composite egg count of a group and the fluctuations in the weight of each of the four lambs within the group.




*Graph* 4.—Giving particulars of the average number of eggs per gram of faeces in group 4, and the weights of individual lambs in the group.

The first table (page 150) gives the numbers of the various species of worms found in each lamb, the ages of the lambs and their survival times on the experimental plots ; and the second table shows a comparison between the four groups, their ages when placed on the plots, their survival period and the numbers of worms found at post-mortem examination in the stomach and small intestine.

*Table* 1. Giving the results of the analysis of the helminthic infestation of all the experimental lambs.

| | Group 1 | | | | Group 2 | | | |
|---|---|---|---|---|---|---|---|---|
| Date when placed in infected pen ... ... ... ... | 24.iii.32 | | | | 5.v.32 | | | |
| No. of lamb ... ... ... | 577 | 578 | 581 | 580 | 575 | 576 | 582 | 583 |
| Date of birth ... ... ... | 3.iii | 3.iii | 4.iii | 4.iii | 25.ii | 25.ii | 9.iii | 9.iii |
| Age in days when placed in pen | 21 | 21 | 20 | 20 | 69 | 69 | 58 | 58 |
| Days survived in pen ... ... | 267 | 252 | 203 | 263 | 123 | 173 | 231 | 203 |
| Date of death ... ... ... | 16.xii* | 1.xii* | 12.x* | 12.xii* | 5.ix | 23.x | 22.xii* | 24.xi‡ |
| Total worm count in stomach | 25,200 | 12,600 | 40,200 | 5,800 | 44,900 | 39,300 | 7,000 | 34,100 |
| *Haemonchus contortus* in stomach ... ... ... | 200 | — | — | — | — | — | — | 12 |
| *Ostertagia circumcincta* and *O. trifurcata* in stomach ... | 6,165 | 2,814 | 8,321 | 1,989 | 21,159 | — | 933 | 8,911 |
| *Trichostrongylus extenuatus* in stomach ... ... ... | 17,323 | 9,534 | 28,663 | 3,695 | 23,741 | 39,300 | 4,667 | 24,495 |
| Larvae other than *Nematodirus* in stomach ... ... ... | 1,512 | 252 | 1,608 | — | — | — | 1,400 | 682 |
| Total worm count in intestine | 33,000 | 11,600 | 56,400 | 6,500 | 47,700 | 72,500 | 6,800 | 54,200 |
| *Ostertagia circumcincta* and *O. trifurcata* in intestine ... | — | — | — | — | — | — | 86 | — |
| *Trichostrongylus vitrinus* in intestine ... ... ... | 24,692 | 8,909 | 38,155 | 2,982 | 47,700 | 61,979 | 3,532 | 40,541 |
| *Cooperia oncophora* in intestine | 2,368 | 1,531 | 7,322 | 1,828 | — | 1,821 | 1,550 | 7,155 |
| *Nematodirus fillicollis* in intestine ... ... ... ... | — | — | 771 | - | — | — | — | — |
| *Strongyloides papillosus* in intestine ... ... ... | 5,280 | 696 | 10,632 | 910 | — | 7,250 | 1,632 | 2,168 |
| Larvae of *Nematodirus* in intestine ... ... ... | 660 | 232 | — | 636 | — | 1,450 | — | 3,252 |
| Larvae other than *Nematodirus* in intestine ... ... ... | — | 232 | 1,128 | 260 | — | — | — | 1,084 |

* Killed at the end of the experiment with the exception of 581 killed earlier because of a broken leg.

TABLE 1—*continued*.

| Group 3 | | | | Group 4 | | | |
|---|---|---|---|---|---|---|---|
| 16.vii.32 | | | | 9.ix.32 | | | |
| 605 | 604 | 606 | 609 | 618 | 617 | 628 | 622 |
| 29.iii | 29.iii | 29.iii | 30.iii | 17.iv | 9.iv | 29.iv | 25.iv |
| 110 | 110 | 110 | 109 | 146 | 154 | 134 | 138 |
| 51 | 46 | 44 | 115 | 33 | 89 | 94 | 98 |
| 5.ix | 31.viii | 29.viii‡ | 8.xi | 12.x | 7.xii* | 12.xii‡ | 16.xii‡ |
| 20,800 | 25,300 | 15,400 | 4,200 | 61,800 | 27,500 | 35,500 | 17,500 |
| — | — | — | — | — | — | 7,000 | 1,650 |
| 7,280 | 5,060 | 6,600 | — | 5,356 | 1,630 | 12,960 | 5,408 |
| 13,520 | 20,240 | 8,800 | 4,200 | 44,084 | 23,670 | 15,540 | 8,342 |
| — | — | — | — | 11,124 | 2,200 | — | 2,100 |
| 27,000 | 15,000 | 38,200 | 33,100 | 29,800 | 52,000 | 20,900 | 34,300 |
| — | — | 1,910 | — | — | 450 | — | — |
| 20,250 | 8,483 | 28,650 | 27,407 | 24,978 | 36,730 | 12,069 | 28,538 |
| 6,750 | 1,462 | 5,730 | 3,045 | 3,034 | 2,930 | 1,214 | — |
| — | 1,755 | 1,910 | — | — | 450 | 3,853 | 4,390 |
| — | 2,100 | — | 1,324 | 1,236 | 4,160 | 2,508 | 1,372 |
| — | 1,200 | — | 1,324 | — | 6,240 | 1,254 | — |
| — | — | — | — | 1,788 | 1,040 | — | — |

‡ Killed in the last stages of the disease.

*Table* 2.—Comparing the helmintic infestation of the four groups and their reactions to disease.

| | | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|---|
| Average of the total number of worms recovered from stomach ... ... | | 20,950 | 31,325 | 16,425 | 35,575 |
| Average numbers of the various species recovered from stomach | Haemonchus ... | 50 | 3 | 0 | 2,162 |
| | Ostertagia ... ... | 4,822 | 7,750 | 4,735 | 6,338 |
| | Trichostrongylus ... | 14,803 | 23,050 | 11,690 | 22,909 |
| | Larvae other than Nematodirus ... | 843 | 520 | 0 | 3,856 |
| Average of the total number of worms recovered from intestine ... ... | | 26,875 | 45,300 | 28,325 | 34,250 |
| Average numbers of the various species recovered from intestine | Ostertagia ... ... | 0 | 21 | 477 | 112 |
| | Trichostrongylus ... | 18,684 | 38,438 | 21,197 | 25,578 |
| | Cooperia ... ... | 3,262 | 2,631 | 4,246 | 1,794 |
| | Nematodirus ... | 192 | 0 | 916 | 2,173 |
| | Strongyloides ... | 4,379 | 2,762 | 856 | 2,319 |
| | Larval Nematodirus | 382 | 1,175 | 631 | 1,873 |
| | Larvae other than Nematodirus ... | 405 | 271 | 0 | 707 |
| Date when placed in infected pens ... | | 24.3.32 | 5.5.32 | 16.7.32 | 9.9.32 |
| Average age when placed in infected pens ... ... ... ... ... | | 20 days | 63 days | 109 days | 143 days |
| Number of lambs which died of parasitic gastritis ... ... ... ... | | 0 | 2 | 4 | 3 |
| Average survival time in infected pens | | 246 days | 182 days | 64 days | 78 days |
| Average number of eggs per gram of faeces at time of death ... ... | | 925 | 5,800 | 6,662 | 16,700 |
| Ratio of worms to eggs, expressed as the number of worms represented by each egg per gram of faeces ... | | 52 | 13 | 6 | 4 |

## Summary of Results Obtained.

### *The Period of Survival of the Lambs.*

Of the earliest group, $2\frac{6}{7}$ to 3 weeks old when placed on infected pasture on the 24th March, none died of the disease and three survived throughout the whole experimental period of 260 days, the fourth having to be killed at the 203rd day because of a broken leg.

The second group of four worm-free lambs were 8 to $9\frac{6}{7}$ weeks old when placed on the infected plot on the 23rd May ; three of the four contracted severe verminous gastritis and either died or were sacrificed in the last stages of the disease, after an average survival time of 182 days.

The third group were $15\frac{4}{7}$–$15\frac{5}{7}$ weeks old when placed in the infected pen on the 27th July ; these quickly developed an acute form of the disease, and although one lived for 115 days, the other three succumbed in 44, 46 and 51 days respectively.

The fourth group of worm-free lambs were $19\frac{1}{7}$ to 22 weeks old when placed in the infected pen on the 19th September, and three either died of verminous gastritis or were sacrificed in the last stages of the disease within 12 weeks of having been placed in the pen.

### *The Weight of the Lambs.*

The rather erratic nature of the weight curves is probably to be accounted for by the low diet and the movements from one pen to another —provisions to ensure that the lambs would graze close to the ground and contract the disease. There is, however, a marked difference between the weights recorded for groups 1 and 2, and for 3 and 4. In group 1 there is evidence of the successful development of immunity, and in group 2 of the development of an immunity which was almost sufficient to resist the disease, but in groups 3 and 4 no evidence of resistance on the part of the lambs is shown in these data.

The one or two individual differences in the weight curves are of interest, No. 618 group 4 shows a particularly marked susceptibility and No. 582 group 2 and No. 609 group 3 a marked individual resistance.

### *The Egg Count.*

The graphs plotted from the composite egg count of the first two groups show the development of immunity as followed by Stoll (1929) in *Haemonchus contortus* and by Sarles (1932) in *Trichostrongylus calcaratus*. In the third and fourth groups death occurred before the establishment of the immunity, and the egg count rises to the point of death.

It is thus seen that these three separate lots of data, the survival period, the weight of the lambs and the egg count, all provide evidence of the ability of lambs to acquire an immunity to parasitic gastritis.

*Differential Worm Count.*

Taking into account the very different reactions of the lambs, the average worm count for individual lambs in the different groups is found to be surprisingly similar : group 1 carried an average of 47,822 worms ; group 2, 76,622 ; group 3, 44,750 ; and group 4, 69,822. There are, however, marked differences between the numbers of certain species, *H. contortus* and *N. filicollis* in particular. Both of these species are large enough and sufficiently characteristic to be singled out fairly easily by the unaided eye and their absence in the instances stated is practically certain. Only 4 lambs carried *H. contortus*, the numbers present being 12, 200, 1,650 and 7,000 respectively. Adult *N. filicollis* were found in only six instances, in numbers varying between 450 and 4,390. These infestations are not confined to any group or age of lamb, but appear to be distributed only according to the individual susceptibilities of the lambs. *Ostertagia spp.* and *Trichostrongylus spp.*, which gave larger counts than any of the others follow one another closely in their distribution in the four groups. *Cooperia sp.* is also fairly evenly distributed, but was apparently absent from two lambs, one in the second group and one in the fourth.

These observations suggest that the immunity is highly specific.

*Correlation between Egg Count and Worm Burden.*

Comparing the egg counts at the time of death with the numbers of worms recovered at autopsy, it is seen that there is a marked absence of correlation. In general the egg count is low in groups 1 and 2 and high in groups 3 and 4 at the time of death of the lambs, but the worm count subsequently proved to be highest in group 2 and lowest in group 3.

*Correlation between Worm Burden and Disease.*

Although the most acute form of the disease developed in the lambs in group 3, these were found to carry fewer worms than those in any other group. Placing the groups in the order of severity of disease, commencing with the most severe, the arrangement is 3, 4, 2, 1, and in order of worm burden, commencing with the greatest number, is 2, 4, 1, 3.

## Discussion.

### (1) *The Demonstration of Acquired Immunity to Parasitic Gastritis in Lambs.*

The clear separation of acquired immunity from age immunity has been a difficult point in helminthological investigation. Where the parasitic worms have been observed to disappear from the host it has not been easy to determine whether previous infection or the age of the animal has been responsible.

There are one or two established instances of age immunity, and latterly the development of acquired immunity has been successfully demonstrated for a few parasites of domesticated and laboratory animals. Stoll (1929) followed the course of an infestation of *Haemonchus contortus* in two lambs at pasture. By a series of egg counts made at weekly intervals he was able to follow the development and the disappearance of infection and to watch the rise of the egg count to a high peak and its subsequent rapid fall, corresponding with the development and disappearance of the symptoms of disease as seen in the members of a flock of lambs suffering from parasitic gastritis under farming conditions. The nature of the immunity, as accurately followed by Stoll, in tracing the disappearance of the eggs from the faeces, or as seen in the clearing up of symptoms in the course of an ordinary outbreak of the disease, might be explained by the age of the lambs rendering them resistant to infection, or by the development of an immunity resultant upon the infection which they had carried, and it cannot definitely be concluded from either of these observations that the disappearance of infection is the result of an acquired immunity.

Ross (1932) obtained results differing from those of Stoll, and considered that age immunity might be an important factor in the resistance of adult sheep to *Haemonchus contortus.*

The experiments here recorded, however, afford an opportunity for comparison between the reactions of worm-free lambs and infected lambs of the same age, grazing the same pasture ; and the resistance which is demonstrated can only be explained by the acquiring of immunity as a result of infection.

The conditions to which the four various groups of lambs were exposed differ from one another in two principal ways, firstly, that the lambs in

each successive group were older than those in the previous group when first they were exposed to infection ; and secondly, that the lambs in each successive group were exposed to heavier doses of larvae at their first admission to the pen, *i.e.*, their infection was acquired at a greater rate.

It is very unlikely that the difference in the age of the lambs at their first exposure to infection would account for the varied reactions which they showed to the heavy rate of infection, as greater age would tend rather to confer a greater power of resistance to the later groups than to make them more susceptible. It is much more probable that while acquiring their infection gradually, the lambs of the first group were allowed sufficient time to develop their immunity previous to the time that the amount of infective material on the ground had become dangerously large. Before the incoming of the second group of lambs the herbage in the pen had been grazed for eight weeks by four lambs (for convenience of comparison this might be stated as 32 lamb-weeks grazing), the accumulation of infective material had presumably been considerable and the lambs of the second group acquired a fatal infection before their immunity had sufficiently developed.

This susceptibility to sudden severe infestation is seen even more clearly in the third group of worm-free lambs, which were placed on the same pasture after it had been exposed to 106 lamb-weeks of grazing. Judging from the egg count and the worm count, this group of lambs acquired in 4½ weeks the same infection as group 2 had taken 13 weeks to acquire, and group 1 taken 15 weeks to acquire; the power of resistance that they were able to develop in that short period was quite inadequate and three of them died within four weeks of their first exposure to infection.

The last four lambs were brought into the pen after it had been exposed to 142 lamb-weeks of grazing, but these lambs did not develop such an acute form of the disease, and survived for a longer period ; this might possibly be accounted for by some diminution in the infective material on the ground resulting from death or removal by grazing of many of the earlier infective larvae ; from the dryer weather being less favourable to larval development, or it might have been due to the development of an age immunity at about this time, these lambs being 6 to 7 weeks older than the previous group when introduced to the infective pasture.

The most significant part of these observations is that throughout the whole experimental period the lambs in the first group resisted the

pathogenic action of the parasites and remained in a healthy condition in the same pen, grazing the same grass and eating the same small amount of concentrated foods as the lambs of the three other groups, most of which perished. And the observation that for part of the time the infection was sufficiently intense to cause the death of three lambs within five weeks, shows the immunity to be of a fairly high order.

This development of immunity, though most clearly and definitely shown in the comparison of the survival periods of the various groups of lambs, is also supported by the observations on the egg counts which are discussed below, and in the weights of the lambs.

(2) *Immunity to the Effects of Parasitism.*

At the conclusion of his review of the available information on immunity to metazoan parasites, Chandler (1932*a*) states that the development of resistance to the effects of helminthic infection constitutes a problem quite distinct from the development of resistance to continued reinfection ; and such appears to be the case in the immunity of lambs to parasitic gastritis. Stoll's work in 1929 dealt entirely with the elimination of *H. contortus* and the prevention of reinfection; disease apparently did not develop.

The complete lack of correlation between the worm burden and the diseased condition of the lambs clearly shows that the immunity concerned in the observations here reported is a resistance to the effects of parasitism rather than to the parasites themselves.

We are still very ignorant as to what the effects of helminthic infection are and of the ways in which these intestinal parasites cause injury to their host, but this observation shows that the infested animals are able to acquire quite a high degree of tolerance to the parasites' harmful action, which is sufficiently potent to cause the death of animals not so protected. Recent investigations by Wells (1931) on *Ancylostoma caninum* in dogs and by Fourie (1931) on haemonchosis in sheep lead these two investigators, from very different premises, to the conclusion that the withdrawal of blood by these two kinds of parasites is in itself sufficient to account for the diseases which they cause. It does not, however, seem reasonable to suppose that the lambs in group 1 could become tolerant to the continued withdrawal of blood over a period of 2 to 3 months, in quantities sufficient to prove fatal in four weeks to lambs which had not acquired this tolerance. Rather does it seem reasonable to suppose the production of some

poisonous substances by the parasitic worms, or some severe irritation to the mucosa to which the host becomes tolerant.

(3) *Effect of the Immunity upon the Parasites.*

The effect of the immune response upon the worms was most clearly shown on haemonchus and nematodirus. The presence of as many as 7,000 *H. contortus* in one sheep and of 4,390 adult *N. filicollis* in another is sufficient to show that the ground carried a tolerably heavy infestation of these two species of parasite ; yet *H. contortus* was only represented in four sheep and mature *N. filicollis* in only six of the whole sixteen which were exposed to the infection. This clearly shows the action of an immunity, eliminating the worms of these two species and preventing fresh infection from taking place. Further evidence of the inhibitory action against Nematodirus is obtained from the observation that ex-sheathed third stage larvae of that species were found in six lambs which were quite free from infection with the adults.

That part of the immune response which results in the elimination of the parasites therefore appears to be highly specific in its action. Species other than *H. contortus* and *N. filicollis* are doubtless also subject to it* to a certain degree (otherwise the lambs in the early groups would have carried much larger numbers than they did), but the elimination and inhibition of development of *Ostertagia*, *Trichostrongylus* and *Cooperia* appears to be much less successful than of *Haemonchus* and *Nematodirus*.

As well as demonstrating the specificity of the immunity these observations also show the presence of differences between the individual lambs, some of which proved to be naturally more resistant than others to certain parasites. Eliminating action on the parasites other than *Haemonchus* and *Nematodirus* appears only to have developed sporadically ; all the lambs carried *Trichostrongylus* in large numbers, all but two carried *Cooperia* and all but two carried *Ostertagia*. *Ostertagia* is regarded as the most pathogenic species and it may be of some significance that the two lambs, 576 and 609, which did not harbour this species, and two others, 582 and 617, which carried fewer of these parasites than the rest, are the four lambs which according to the weight chart resisted the disease

* Observations on natural outbreaks of parasitic gastritis where *Ostertagia* has particularly been concerned have shown that towards the end of an outbreak the number of worms to be found in the stomachs of recovering lambs is not excessively high.

most successfully. On the whole, the relative conditions of the individual lambs as shown by the weight chart compares closely with the relative numbers of parasites afterwards recovered ; but it corresponds even more closely with the relative numbers of *Ostertagia* and bears out the general view that this species is particularly pathogenic.

(4) *Effects of Immunity upon the Worms in Inhibiting Egg Production and Arresting Development.*

A comparison between the egg count and the worm count confirms the observations of Chandler (1932b) (in *Nippostrongylus muris*), Sarles (1929) and McCoy (1931) (in *Ancylostoma caninum*) who observed that the development of immunity has an inhibitory action upon the egg production of the worms in immune animals. The lambs in group 2 carried the largest number of worms but passed relatively few eggs at the time of death, while those in group 3 carried the fewest worms but showed a much higher egg count at the time of death. This shows that although the egg count may serve as a good indication of the progress of the disease it cannot be taken as a reliable indication of the numbers of worms which a lamb may be carrying. The inhibitory action on egg production is very considerable, and as shown in Table IV may be sufficiently powerful to reduce the egg-production of the worms to one-thirteenth of what it otherwise would be.

Evidence was also obtained showing the action of the host's resistance in arresting the development of immature worms. This was seen to be particularly marked in one or two instances of infestation with nematodirus ; six lambs carried numbers of third-stage hookworm larvae of these worms ranging up to 3,252 without carrying any of the mature worms, and one lamb which carried only 450 adult nematodirus carried 6,240 larvae.

Scott (1928) recovered undeveloped third-stage larvae from the organs of experimental rats, cats and dogs several weeks after administration of the infective larvae, and it is probable that the nematodirus larvae found in these lambs had been in the intestine for some time, as comparatively few larvae belonging to other species were found. This inhibition of growth has been observed in *Nippostrongylus muris*, *Ancylostoma braziliense*, *Ascaridia lineata* and *Haemonchus contortus*.

Stoll (1929) found that the *H. contortus* from his immune lambs were smaller than the average size and measurements were therefore made to

ascertain whether the worms (having a reduced egg output) in the resistant lambs in the experiment here reported were of a smaller size than those in susceptible animals, and whether any difference was to be found in the percentage of females which contained eggs. For this purpose worms from the stomachs and intestines of two lambs from each group were mounted on slides and examined under the microscope, measurements being made by the use of the Camera Lucida. The slides were traversed in the usual systematic way until 10 females of all the various species concerned from each of the eight lambs had been examined and measured. On subsequent analysis of the results no difference could be found, however, either in the sizes of the worms or in the percentage of females containing eggs, between those from resistant lambs of the first group or the comparatively resistant ones of the second group, and those of the susceptible lambs of the third and fourth groups.*

*The Time Required for the Development of Immunity.*

The development of resistance appears to be a slow process extending over several weeks. In McCoy's (1931) experiments with repeated doses of *Ancylostoma caninum* in dogs the fall in the egg count occurred somewhere between the eighth and the eighteenth week of infection. The egg output of Stoll's two lambs infected with *Haemonchus contortus* reached its maximum 14 and 15 weeks respectively after the first exposure to infection. Sarles (1932) observed the development of immunity to *Trichostrongylus calcaratus* in rabbits at the 14th and 15th weeks of infection, and in the observations here reported the drop in the egg count occurred at the 18th week in group 1 and at the 13th week in group 2. The lambs in groups 3 and 4 did not survive long enough for the immunity to develop, and it is interesting to note that although the lambs in group 2 suffered so severely from the effects of the parasitism that three of them died, the resistance is registered in the marked drop in the egg count in the same way that it is in the lambs which survived, suggesting that the part of the immune response of the host, which inhibits the production of eggs by the parasites, develops earlier than the resistance to the harmful effects of the infestation. It should be noticed, however, that McCoy (1931) observed

* Observations since made have shown that the numbers of eggs in the female worms present in resistant sheep may be small in comparison with the numbers carried by females in non-resistant sheep.

a reverse order of events, *i.e.*, a development of resistance to some of the effects of parasitism before any diminution in the egg count occurred; the haemoglobin content of the blood of his experimental dogs began to improve before the maximum egg production had been obtained by the parasitic worms (*A. caninum*).

*The Possibility of a Self-Limiting Factor Operating in Worm Infestations.*

It will be noticed that the numbers of parasitic worms recovered from the various members of the four groups of lambs approximated each other fairly closely in spite of the very different reactions shown by the hosts. Group 1 carried a total of 191,300 worms, group 2, 306,500, group 3, 179,000, and group 4, 279,300. The immunity reactions of the lambs have presumably played some part in bringing this about, but a closer correspondence between the numbers of parasites and the state of the resistance of the lambs would have been expected, and greater differences between the total counts.

There are other instances which appear to suggest that a parasitic infestation may be limited by something particularly concerning the parasite and quite apart from the resistance of the host. The observation that the presence of one individual *Taenia saginata* in man inhibits super-infection, but that susceptibility returns as soon as the worm is expelled might be explained by supposing that some excretion of the worms is inimical to the development of other worms of the same kind. And the well-known instance of the comparatively low grade infestation of *Heterakis gallinae* which is usually found in spite of the heavy reinfestation to which the host is continually exposed, may be explained in the same way; Graybill (1921) found it impossible to produce a heavy infection by feeding numerous larvae to susceptible chickens. The observations of Hill (1926), Sarles (1929), and Winfield (1933) that egg production is lower in heavy infestations than in light ones is probably due to something of the same kind.

The presence of some growth stimulating substance which becomes completely used up in heavy infestations has been suggested as an explanation; but whatever the reason may be, the evidence indicates the operations of some factor concerning overcrowding, and the extent of the infestation appears to be limited to a certain extent by the worms themselves.

*Natural Immunity.*

Some evidence of inherent individual resistance to parasitism has been encountered during the course of much of the work on immunity to parasitic worms, and two good instances have occurred while making these observations ; one of outstanding individual resistance and one of marked susceptibility. Lamb No. 609 in group 3, as shown by the weight curve, possessed a much greater resistance than the other three members of its group, and lamb 618 in group 4 was apparently much more susceptible than the other three lambs in its group.

## Summary and Conclusions.

Four lambs introduced to infective pasture early in life acquired an immunity to parasitic gastritis, the immunity being sufficiently strong to maintain them in good health under conditions of repeated reinfestation, heavy enough to lead to the death of ten lambs of a like age, but not so protected, within periods varying from $6\frac{2}{7}$ weeks to $29\frac{4}{7}$ weeks. At post-mortem examination the protected lambs were found to carry more worms than many of the lambs which died, although they had been observed to disseminate only one-thirteenth the number of eggs of some of those fatally affected. The infestation of individual lambs in the various groups showed marked differences, amounting in some instances to the presence of a large number of worms of one species in some lambs and the total absence of worms of that species from others.

The observations lead to the following conclusions :—

1. Lambs are able to acquire an immunity against parasitic gastritis powerful enough to protect them against a rate of reinfection at pasture that is sufficiently great to result in the death of unprotected lambs in less than seven weeks.

2. This immunity operates against the acquiring of infection, but also in enabling the lambs to resist the injurious effects of the infection. Resistant animals may carry more parasites than are carried at the time of death by non-resistant animals.

3. The immunity is acquired slowly and does not appear to become firmly established for 18 weeks. A gradually acquired infection leads to immunity while a quickly acquired infection leads to death where the final number of parasites is the same in both instances.

4. The immunity is specific and operates more powerfully on *N. fillicolis* and *H. contortus* than on *Ostertagia, Trichostrongylus* and *Cooperia*.

5. The immunity has an inhibitory influence on egg laying and on the development of young worms; the egg output of the worms may be reduced to one-thirteenth of the normal.

6. There are marked differences in the reactions of individual lambs, instances of marked resistance and of marked susceptibility having been observed.

7. The symptoms of parasitic gastritis are due to something more than the abstraction of blood by the worm.

8. The part of immunity which inhibits the production of eggs by the parasites develops earlier than the resistance to the harmful effects of the infestation.

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*Journal of Helminthology, Vol.* XII, *No.* 3, *June,* 1934, *pp.* 165–176.

# Ascariasis and Vitamin A Deficiency in Pigs.

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In 1927 and 1928 Hiraishi published some observations which he had made on Ascariasis in swine in the absence of Vitamin A. He claimed that he had been successful in overcoming the resistance of the pigs to the human strain of *Ascaris lumbricoides* by lowering the vitamin A content of the diet and that he was able to produce much heavier infestations with *Ascaris lumbricoides* of swine origin in A-avitaminosed pigs. As all other factors were apparently controlled, he draws the conclusion that vitamin A has a direct bearing on the susceptibility of the pig to infestation with round worms. Unfortunately these publications are not readily available in England and only summaries of results have been available to the present writer. But from the data given and in comparison with the effect of the vitamins on *Ascaridia* (Ackert *et alia*, 1929 and 1931 a & b) and on hookworm (Foster & Cort, 1931 and 1932), these conclusions would seem to be justified.

Shortly after reading these papers, two young sows, just weaned, were placed at my disposal by Professor Leiper for experiment. They were litter mates aged 9 weeks. When they were first taken from the mother and arrived at the Institute of Agricultural Parasitology, faecal examinations were made and a marked infestation with *Ascaris* and a very slight infestation with Whipworm were noted. It is not known therefore exactly when they became infested ; neither is the actual degree of infestation known. But from certain data it was reasonable to infer that they carried very similar infestations. From egg counts it was found that they both had about 6,000 eggs per gramme of faeces and furthermore, being litter mates, they had been kept under identical conditions, in the same sty and the possibilities of picking up Ascaris infestation were equal. The experiment about to be described therefore, was built on this assumption.

When the pigs arrived at the laboratory, considerable care was taken to avoid re-infestation. They were separated, kept in adjoining sties which had been sterilized with a blow lamp. These sties were cleaned out daily. At the age of 12 weeks, one sow—E.2.—was put on a diet deficient in Vitamin A. consisting of steamed casein, tapioca meal and white maize, while limestone grit was added abundantly. This diet was balanced and was adequate in all respects other than the vitamin A content. At intervals a few drops of irradiated ergosterol were given to prevent rickets as the pigs were reared indoors. The other pig—E.1.—was fed the same basal ration with the addition of cod liver oil. Later both pigs were given a mangold daily. Both the animals throve on this diet.

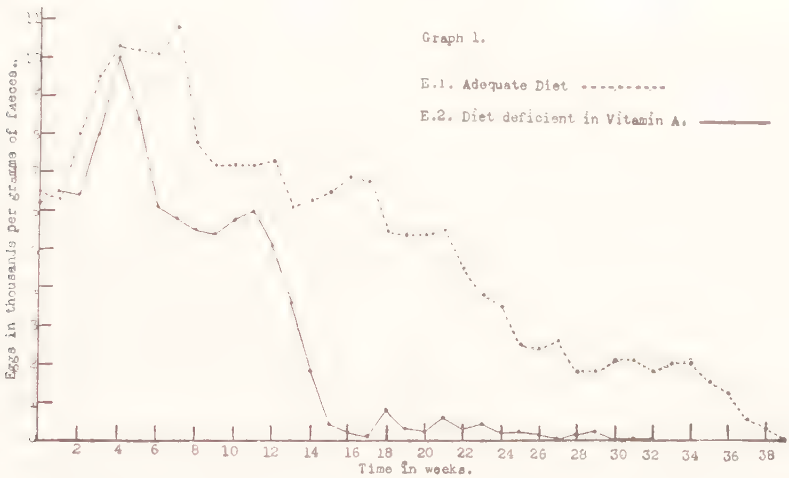
Pigs do not readily show the effect of the absence of vitamin A unless the deficient diet be fed very early after birth. Sow E.2. continued to grow but the absence of the vitamin was apparent in the sclerotics of the eye. These were thick, opaque and of a decided yellow colour. The skin too was dry and scaly, thus showing definitely that she was reacting to the deficiency. Both pigs became very fat immediately after they were put on these diets but this was probably due to the inclusion of tapioca meal, which is an excellent fattening food for swine. Sow E.1. throve on this diet and grew healthily.

A striking difference was seen in the worm burden of the two pigs. The course of the infestations was followed by egg counts by the Stoll dilution method. Three egg counts were made each week from each pig and the average of the counts calculated. Thus in the graphs, only one figure is given for each pig for each week. This represents the average count. In this way was eliminated in some degree, the errors due to individual variation.

Morgan (1931) has previously followed the course of infection in Ascariasis in three pigs, which had been fed with larvae obtained from the lungs of another pig which had died of Ascaris pneumonia. He found that the egg-laying life of *Ascaris lumbricoides* was about 17 weeks but that during the last few weeks a low egg count prevailed and most of the eggs passed were infertile. Hence their useful egg-laying life is limited to a shorter time.

In the main the present results bear out those of Morgan, though the infestations lasted much longer. As has already been pointed out, the worms were passing a fair number of eggs before regular counts were made

and infestation must therefore have taken place some weeks earlier, when the pigs were very young. The count steadily increased and reached a peak about 4 weeks after regular counts were instituted (Graph 1). After this there was a regular and gradual drop. To take the pig (E.2.) on deficient diet first. This drop in egg count was regular and rapid and by the 15th week the counts had become negligible. For a considerable time afterwards, a very low count was maintained and such eggs as were found, were infertile. A different state of affairs was observed in the other pig (E.1.) which was fed an adequate diet. The peak of this infestation occurred a little later but this was followed by a regular drop. This fall




was, however, slow. By the end of the 15th week, when her companion was passing less than 1,000 eggs per gramme of faeces, this sow was still passing over 6,000 eggs and she continued to do so for some time afterwards. It was not until the 37th week that the drop became appreciable. As was expected, during the last few weeks of life, all the eggs that were passed were infertile.

Morgan has suggested two theories, both of which would account for the constant appearance of infertile eggs at the end of the infestation. Either the males have a shorter life than the females or the supply of sperm that they provide is insufficient to fertilise all the eggs of one female. Whichever supposition may be correct, it is obvious that the

supply of sperm and eggs available in the E.1. pig was more adequate than in E.2. For here, not only was the egg count higher all the time, but the useful egg laying life of the worms was considerably longer. Infertile eggs did not appear in large percentage until the 28th week as compared with the 14th week in E.2.

When no Ascaris eggs were found, by the Stoll dilution method of examination, the faeces were examined by the flotation technique, using saturated sugar solution to float up the eggs. This method was used until both pigs had given negative results for 6 weeks. They were then re-infested in the following manner. A culture of embryonated eggs, showing 96% infective eggs, were used. 1cc. of this culture was fed daily to each sow, mixed with the evening meal, for 10 days. Three weeks after the first feed a few infertile eggs were demonstrated in the stool by the flotation technique. The following week, these eggs were sufficiently numerous to be counted by the Stoll dilution method : examinations continued for 7 weeks during which time both rose slightly, giving 800 (E.2.) and 1,000 (E.1.) eggs per gramme of faeces. After that the worms must have been discharged for no more eggs could be found in the faeces. The infestation was repeated and the same result was obtained. In both pigs, eggs were found for 4 weeks, after which they all disappeared. This result points to the establishment of an immunity, but whether this immunity is due to the age of the sows or to the earlier infestation is not evident from the data available. Ransom (1922) believed that a complete immunity was established at the age of 4 months, but in view of the infestations described above and of Morgan's successful infestations at the age of 10 months, this would seem to be rather too early. It may be that this immunity is due, in some measure at least, to the previous infestation.

The observations made from these first infestations (shown graphically in Fig. 1) do not bear out those of Hiraishi, for here we have the disease less pronounced and running a shorter course, in the absence of vitamin A. It was thought that the reason for it might lie in the following fact. Both pigs were being fed a normal and adequate diet all through the important migration stages and until the infestations had become well established. Therefore a repetition was decided upon with the single difference that the pigs should be fed the deficient diet before infestation occurred. For this purpose a litter of 8 pigs was obtained from a clean sow. They were weaned at 8 weeks when they were sturdy and strong. They were then

divided into two groups of 4, closely matched for weight and sex. At this time they had no parasites of any description though faecal examinations were made for three weeks before weaning. They were fed the basal ration of white maize, tapioca meal and casein. Group 1 (containing pigs numbers F.1., F.4., F.5., and F.9.) was given in addition, a little cod liver oil daily while group 2 (containing pigs numbers F.3., F.6., F.7. and F.8.) had an equivalent amount of olive oil. The quantity increased as the pigs became older. Thus group 1 was being fed a complete diet as the cod liver oil contained the necessary vitamin A and group 2 was still deficient in the vitamin. The addition of olive oil, however, made up the extra fat. At first group 1 refused the food and the weights of the pigs accordingly fell but after 10 days, they took it without trouble and from that time continued to grow. They were all fed these diets for 5 weeks and all the pigs were weighed each week. By this time group 2 was beginning to show the effects of the avitaminosis just as E.2. did but more markedly. All were then fed infective Ascaris eggs. An excellent culture was available, containing over 90% embryonated eggs and the larvae were in good condition as was evidenced by their active movements inside the shell. These were fed as before, mixed with the food, but at the mid-day meal this time. 2 c.c. of this culture was given to each group for 10 days. After 2 days the pigs began to lose their appetites and two (F.9. and F.5.) coughed for 4 days. The extra-intestinal migration of Ascaris larvae that is necessary before they can settle down in the intestine and mature, involves their penetration into the blood vessels. The larvae are carried along with the blood stream, growing all the time, through the liver to the right heart and thence to the lungs. Here they are filtered out, being too large by now to pass through the capillaries. They therefore break out into the alveoli and find their way to the bronchi, up the trachea into the mouth. Finally they are swallowed and on arrival in the intestine a second time, they can settle down. In heavy infestations, severe pulmonary symptoms may result from this lung invasion. The whole lungs may become ecchymotic and oedematous with a marked fibrinous exudate into the respiratory tracts, which consists of erythrocytes, leucocytes, migrating larvae and desquamated epithelial cells, together with a dense mass of well defined fibrin threads. The result is a profound lobar pneumonia. In severe cases the whole of the lungs may be completely solid. It was in the hope of avoiding this complication that the chosen

method of feeding was used. The pulmonary symptoms were spread over a long time and were less acute than those resulting from an excessively large dose given all at one time. In three weeks time eggs could be demonstrated in the stools of the pigs of group 1 by floating them up with sugar solution. The number increased rapidly so that by the next week egg counts could be made by the Stoll dilution method. In group 2, however, no infestations could be found. They were examined very carefully for some days, after which they were re-infested. On 7 successive days 2 c.c. of the same Ascaris culture was administered with the mid-day meal and as a result of this infection, all the pigs developed the worms. Within two days they were noticed to lose their appetite and to develop a frequent cough. Respiration in two of them became noticeably difficult and rapid. These symptoms subsided after 6 days

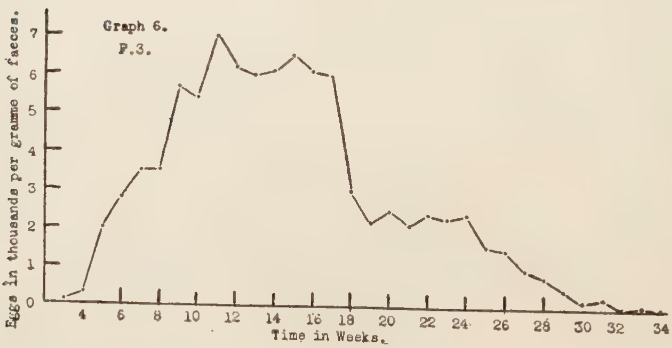

when the animals made an uneventful recovery. The symptoms indicating migration through the lungs were much more marked in this group and seemed to point to heavy infestations having occurred. Shortly after these symptoms had passed, all the animals developed an intense diarrhoea which persisted for about 3 days. During these weeks it was obvious that the pigs were not thriving but after the diarrhoea had subsided, their improvement was very marked. They were all weighed each week and both groups made excellent progress, though those given an extra ration of cod liver oil somewhat outstripped the others.

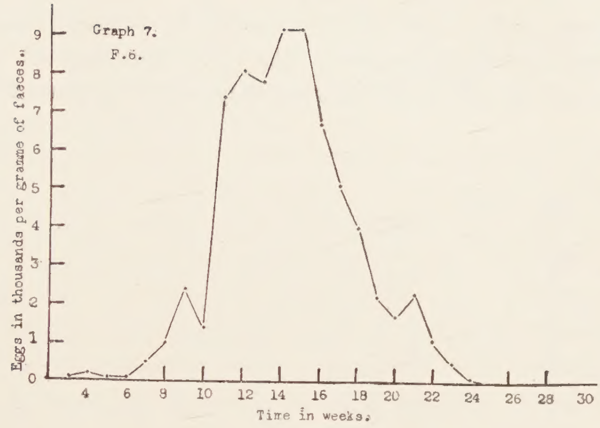
The course of these infestations was followed by means of egg counts. Owing to the large number of experimental animals, it was only practicable to examine each animal once a week and hence in the graphs (Graphs 2–9), which describe this experiment, certain weekly fluctuations will be apparent.

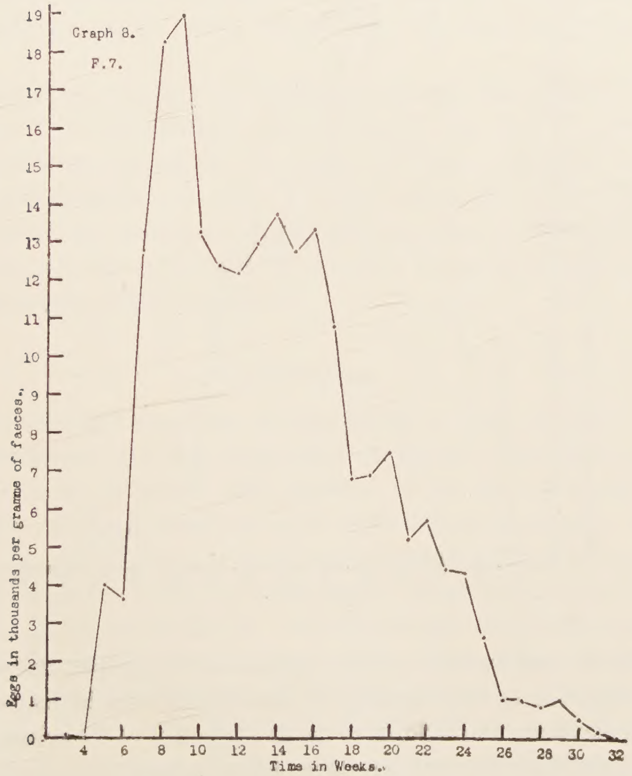

Graph 3.
F.5.
Eggs in thousands per gramme of faeces.
Time in Weeks.


Graph 4.
F.1.
Eggs in thousands per gramme of faeces.
Time in Weeks

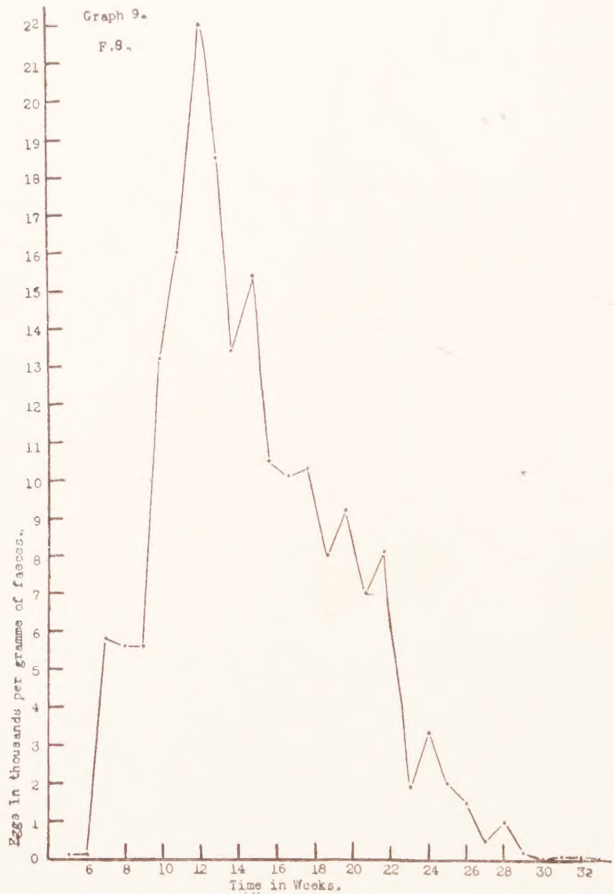

Graph 5,
F.9.
Owing to the rapid rise, the scale of this graph is divided by two.
Eggs in thousands per gramme of faeces.
Time in Weeks.


Graph 6.
F.3.
Eggs in thousands per gramme of faeces.
Time in Weeks.


Graph 7.
F.6.
Eggs in thousands per gramme of faeces.
Time in weeks.


Graph 8.
F.7.
Eggs in thousands per gramme of faeces.
Time in Weeks.

Pigs F.9. and F.8., weighing $24\frac{1}{2}$ and $20\frac{1}{2}$ lbs. respectively were the two small pigs of the litter while F.1. and F.7., weighing 34 and 36 lbs. respectively were the large ones. The other four pigs all weighed to within an ounce or two of 28 lbs. when they were first given the infective eggs.




It will be seen by even a casual glance at the graphs that there is no great difference in the intensity of the infestations indicating that the vitamin plays any marked role in the development of Ascariasis. The two small pigs of the litter both developed heavy infestations, particularly F.9.

(on adequate diet). Here the infestation was so great as to make it necessary to draw the graph at half the scale used for the other animals (Graph 5). The two big pigs developed almost equal infestations, judging by the number of eggs passed out in the stool. The other four pigs all developed much lighter infestations.

Next as to the time factor. These infestations ran a more rapid course than had those in E.1. and E.2., but even so they were longer than those described by Morgan (1931). F.6. on deficient diet became " clean " first, the disease lasting 21 weeks (Graph 7) : the other infestations lasted a few weeks longer. It was noticed once again that those pigs fed adequate vitamin A retained their worms for longer time than the others. In F.9. the disease ran an interesting course : there was a rapid steep rise followed by an equally rapid and steep fall, the peak occurring 13 weeks after the eggs first appeared in the stool. Eggs finally disappeared after 31 weeks.

Most of these graphs show a peak at about the same time, though F.3. (Graph 6) shows a more or less flat plateau, lasting from the 11th–17th week.

Not all the faeces from each individual animal were collected all the time, but at times Ascaris were found passed in the stool of a particular pig. For instance, during the 21st week, F.9. passed 11 adult worms in one stool, and on another occasion F.7. lost a worm during the 17th week. These females were examined for eggs and they were invariably found to contain only infertile eggs, while in the male the stock of sperm seemed to have been completely used up.

## Conclusions.

These results are somewhat disappointing in that they provide no obvious conclusions that can be drawn from them. Considering the first experiment alone, it would seem possible to say that the presence of vitamin A in the diet is beneficial to the well-being of the Ascaris. which is in direct contrast to the conclusions of Hiraishi. But turning to the second experiment in which a larger number of animals has been used, no definite conclusion can be drawn. On the average, the infestations in the group provided with adequate vitamin, were the heavier. But marked infestations eventually took place in all the animals. One interesting feature appeared in the group fed deficient vitamin, where all four animals resisted the first attempt at infestation and yet at the second they all

became sufficiently heavily infected to show the symptoms of Ascaris pneumonia. The sudden attack of diarrhoea which followed the subsidence of these symptoms, may have been due to the arrival in the intestine of a large number of worms producing toxic substances. It is highly probable that a quantity of them were discharged at that time.


## Acknowledgments.

These experiments were carried out at the Institute of Agricultural Parasitology during my tenure of the Lawrence Research Studentship and with material assistance from a grant to Professor Leiper from the Medical Research Council.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